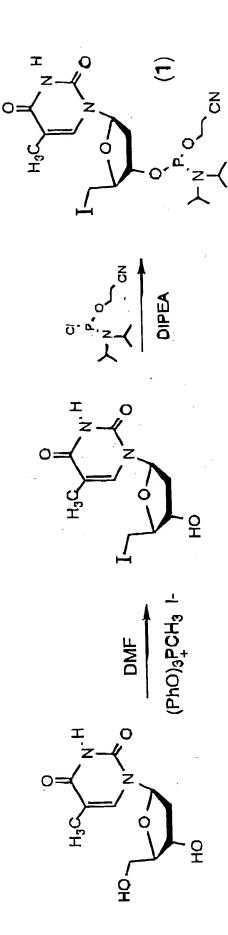


Upstream
oligonizatide downstream
oligonizatide template

5'
Poly nucleotide template
31

Fig. 1

Fig. 2



Tig. 20

## (a) ligation of ssDNA

## ligation of duplex DNA

GATCAGGT TTCACGAGCCTG

+
CTAGTCCAAAGTGCTCGG

TTGTACGCT GGAT GCA

GATCAGGT. TT CACGAGCCTG

TTGTACGCTGGATGCATCCAGCGTACTTTCATGCGACCTACGTAGGTCGCATGT

## one pot ligation / cyclization of ssDNA

GTTTTATACAAAACCTGGCA

TT CAGCAAAATATGT S TTTTGGACCGTTGGT

C + C

CTGCTTCACTAGT S TCAGGAGACTGTTCAG

AGTGATCAAGTCCTCTGA

T CAGCAAAAT AT GT.TTTGGACCGTTGGTT
C
T
C
T
CTGCTTCACTAGT.TCAGGAGACTGTTCAG

(b)	rxn. type	conversion	isolated yield
	ssDNA ligation	>90%	44%
	duplex ligation	75%	36%
	ligation/cyclization	>90% (1st step) 50% (2nd step)	20%

Fig. 4

exonuclease / hydrolysis susceptibility

5-GATCAGGTpaTTCACGAGCCTG-3

endonuclease susceptibility

T TGTACGCTGGA TGCAPTCCAGCGTACTTTCATGCGACCTPACGT AGGTCGCATGTT

template for replication / transcription

STAATACGACTCACTATA SATTATGCTGAGTGATATCCTGCCTATTCCGAGCACTT,TGGACTAG

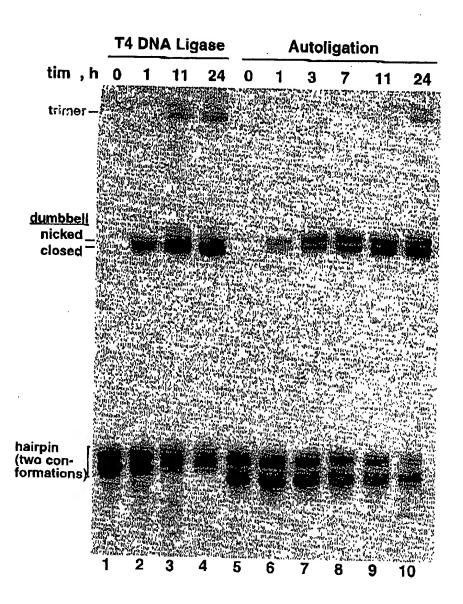


Fig. 6

dumbbell

5' bridging phosphorothloate duplex:

TTGTACGCTGGATGCASTCCAGCGTATCTT
TCATGCGACCTSACGTAGGTCGCATAGTT

all phosphodiester duplex:

Nsi I
TGTACGCTGGATGCATCCAGCGTATCTT
TCATGCGACCTACGTAGGTCGCATAGTT

hairpin

Fig. 7

linear probes MUT target 3°CCACCACCCGAGGCAGC 5'MM target 3 CCACCACCGAGGCCGCCACACCCATTC5 3'MM target \*CCACCACCGAGGAAGCCACACCCATTC\* 3'CCACCACCGAGGCAGGCACACCCATTC5' MMM target cyclization probe MUT target 5'MM target \*CCACCACCGAGGCCGCCACACCCATTC 3'MM target 3 CCACCACCGAGGCAGCCAAACCCATTC5 MMM target

Fig. 8

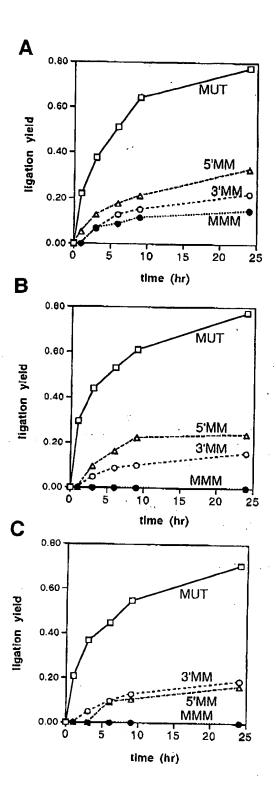


Fig. 9

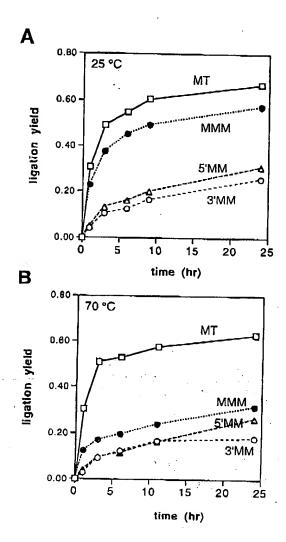
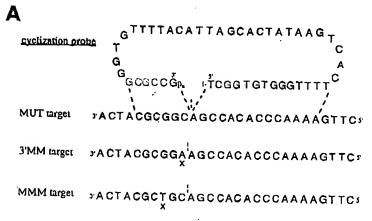


Fig. 10



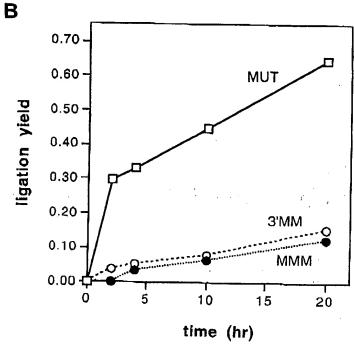
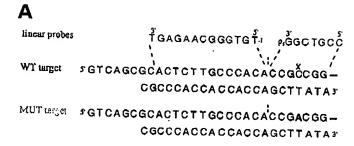
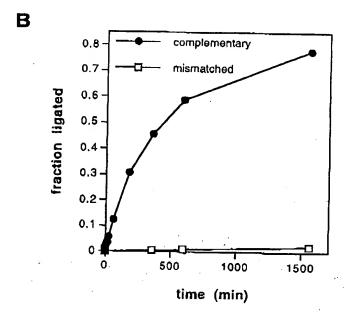


Fig. 11





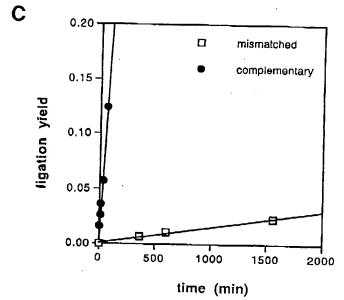


Fig. 12

Probes 3'TGAGAACGGGTGT5' -3'GGC\GCC\(X=G,T)\)
WT target
5'GTCAGCGCACTCTTGCCCACACCGGCGCGCCCCACCACCACCAGCTTATA3'

Fig. 13

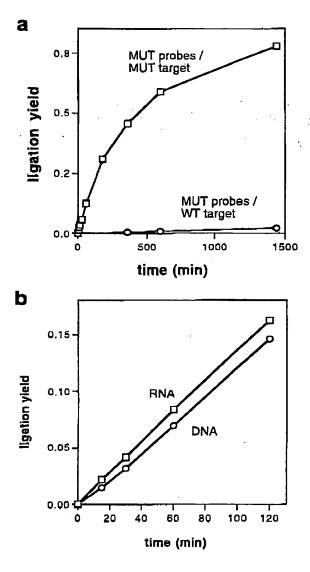


Fig. 14

	temperature (°C)	[target] (nM)	fold turnover	
			no cycling <sup>a</sup>	cycling <sup>b</sup>
	22	1	24	
		10	1.6	1.0
		100	1.0	1.0
	27	1	13	14
		10	1.6	3.0
		100	1.2	1.2
	32	1	40	51
		10	4.6	4.7
		100	2.3	2.3
	37	1	30	44
		10	5.9	. 6.2
_		100	2.2	2.2

<sup>&</sup>lt;sup>a</sup>Simple incubation of 10  $\mu$ M probes with target for 24 hr followed by gel electrophoresis and quantitation of ligated product. <sup>b</sup>24 hr of thermal cycling (30 min at temp. shown followed by 45 sec at 95 °C).

(6)

(Q)

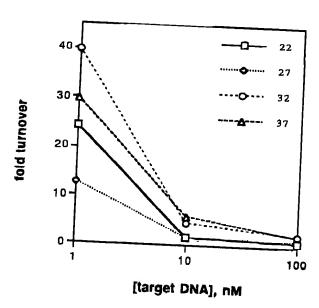


Fig. 15

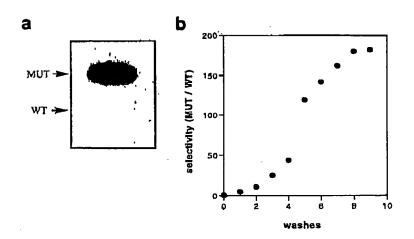


Fig. 16

## universal probe (FAM label)

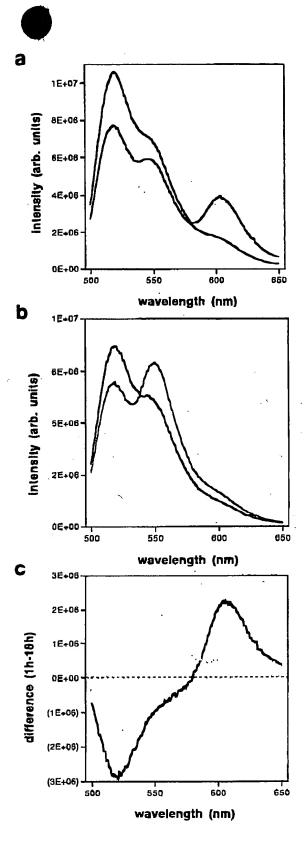


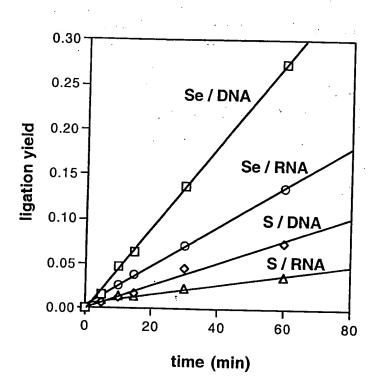
Fig. 18

3'TGAGAACGGGTGT5' scGGCXGCC5' probes (X=G,T) WT target 

MUT target

5'GTCAGCGCACTCTTGCCCACACCGACGCCCCACCACCACCAGCTTATA3'

MUT RNA target 5'GCGCACUCUUGCCCACACCGACGCGCCC3'



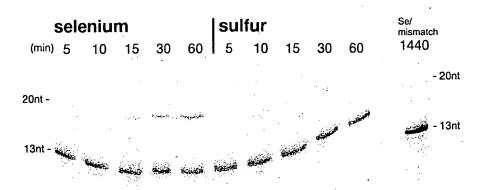


Fig. 21